with low and high loading of primary DNA molecules and the resulting difference in the protein detection. As shown in the schematic illustration of panel (a) polylysine coating of the PDMS support results in an increased loading of DNA oligomer codes.

[0167] In particular, the DNA-loading density is estimated to be 6×10^{13} molecules/cm^2 in our experiments, an order of magnitude higher than typical loading densities on amino-silane coated glass slides. As a result, the protein detection sensitivity was improved by an order of magnitude, and the dynamic range was increased to 4 orders of magnitude, as compared with 2-3 orders of magnitude for the small-molecule amine (i.e. amino-propyl-triethoxyl silane, APTES) functionalized glass surface. Exemplary results of this comparative analysis is illustrated in FIG. **14** Panel (b) detection of three human cytokines (IFN-γ, TNF-α, and IL-2) using substrates coated with amino-silane and polylysine, respectively is shown.

Example 3

Barcoded Chip with ELISA-Like Sensitivity

[0168] A series of experiments performed by the applicants showed that a barcode chip integrated with DEAL technology renders a high density array for multiplexed protein measurements. Moreover, the DEAL barcoded chip also demonstrates a marked improvement in sensitivity as compared to conventional pin-spotted microarrays.

[0169] In particular, a side-by-side comparison study was performed by running DEAL assays on three cytokines under identical conditions. Using the microchannel-guided flow patterning method, a glass slide was patterned with DNA oligomers A, B, C and a blank control O. Each bar was 20 μm in width. The DNA solutions were all 50-100 μM. The pin-spotted array was printed at the Institute for Systems Biology at 100 μM concentration. The typical spot size was 150-200 μm. Six sets of spots were printed corresponding to oligomers A, B, C, D, E, and F. Poly-l-lysine coated slides were used for both types of arrays.

[0170] Before the DEAL assay, the capture antibodies were conjugated to DNA oligomer codes as follows: A' to IFN-γ, B' to TNF-α and C' to IL-2. Protein standards were diluted in 1% BSA/PBS solution at concentrations ranging from 1 fM to 1 nM. The incubation time for each step (blocking, conjugate hybridization, sample binding, detection-antibody binding, and fluorescent-molecule binding) was 30 min. The bar width was 20 μm.

[0171] The results are illustrated in FIG. **15** wherein immunoassays run on DEAL barcode arrays is shown. In particular, as illustrated in Panel (a) detection of three human cytokines (A: IFN-γ, B: TNF-α, C: IL-2, O: negative control) was proven to be concentration dependent. In the illustration of Panel (a) the bar-code array has a sequence of ABCOABCO-ABCOA (herein, "0" denotes that no 1° DNA was flowed in such microchannel). This data show proteins can be detected at concentration as low as 1 pM. Concentration dependence is indicated by the diagram of Panel (b) where quantitation of fluorescence intensity is plotted versus TNF-α concentration. The line profile for the results obtained with 1-pM protein sample as indicated in Panel (a), is shown in the diagrams of Panel (c).

[0172] As a further comparison, the sensitivity obtained in ELISA assays (using antibody pairs and protein standards from eBioscience) is projected to be ~10 pg/mL (0.8 pM) for TNF-α. Therefore, those experiments show that the DEAL barcode array combines ELISA-like sensitivity with a high degree of multiplexing for protein measurements.

[0173] In addition, the TNF-α detection sensitivity of the DEAL barcode arrays was higher and the projected sensitivity limit was better than 1 pM, as compared to 10-100 pM for conventional microarrays as illustrated in the comparative assay performed under the same condition using a conventional pin-spotting method of Panel (d) further illustrated in the comparative Example 6 below. These results confirmed that the barcoded chip has much higher sensitivity and increased linear range for protein measurements, as compared with a conventional assay.

Example 4

Use of a Barcoded Array for DNA Detection

[0174] A barcoded array was used in a bio assay for detection of DNA. In particular, a polynucleotide (DNA) was patterned on a substrate and used to detect a complementary polynucleotide in a sample. The results illustrated in FIG. **16** show that the patterned DNA oligomers exhibit a high affinity for binding their complementary strands.

[0175] In particular, in FIG. **16** panel A, fluorescence images are reported taken before and after hybridization of an A' strand to its Alexa 532 labeled complementary stand. Three different strands of DNA oligomers, nonfluorescent A, Alexa 532 labeled B (red) and Alexa 635 labeled (dark green) were flow-patterned on a polyL-lysine slide to form this barcode chip. "0" denotes a non-patterned channel for bland control. After applying the Alexa-532 labeled A' molecule s (its concentration is 1 nanomolar, these DNA molecules are complementary to the surface bound A stands), a clear and strong green fluorescence band emerges, indicating highly effective and specific sensing of A' DNA molecules.

[0176] The line profile of fluorescence intensity across the whole set of bar-code array is shown in FIG. **16** Panel B. In the illustration of FIG. **16**, A' is the target polynucleotide that was added into sample b and detected by fluorescence change in the location indicated by an asterisk.

Example 5

Use of Barcoded Array for Protein Detection

[0177] A barcoded array assembled as disclosed herein was used for protein detection according to an experimental approach developed by the applicants.

[0178] In particular, applicants developed a multiplexed assay of 12 plasma proteins using DEAL barcode arrays. In a first test, the level of cross-reactivity of each antigen with DEAL stripes that are not specific to that antigen was assessed. DNA-encoding capture antibodies and biotinylated detection antibodies for all 12 antigens were used as usual, but a distinct antigen (10 nM) was added to each assay lane. Cy5-Streptavidin (red-fluorescence tag) was run as usual to visualize the extent of analyte capture.

[0179] The reference marks (DNA strand M) were visualized in all lanes with fluorescent green Cy3-M' DNA molecules. The 12 proteins showed a negligible extent of cross-talk. In a second test, assays were performed on serial dilutions of all 12 proteins on the DEAL barcode chip in view of the limitation imposed by the particular devices used, each allowing a maximum of 12 parallel assays to be executed. In the specific experimental approach of choice for this setting 6